Antibody Characterization Report for Rho GDP-dissociation inhibitor 1 (Rho GDI 1)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Rho GDP-dissociation inhibitor 1

Recommended short protein name: Rho GDI 1

Alternatives protein names: RhoGDI, Rho-GDI alpha

Gene name: *ARHGDIA*

Uniprot: P52565

We are a third-party organization with the mission to characterize commercial antibodies for all human protein through open science [1]. This report guides researchers to select the most appropriate antibodies for Rho GDI 1. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Rho GDI 1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. A HEK-293T *ARHGDIA* KO line is available at Abcam and was used in this study. HEK-293T expressed appropriate Rho GDI 1 protein level as determined by searching PaxDB [3].

The authors do not provide an assessment of the quality of the tested antibodies as their respective performances are limited to our finite experimental conditions. The readers should interpret the present findings based on their own scientific expertise. The authors acknowledge that an antibody that demonstrates specificity in the stated test conditions can be suboptimal in a different experimental format or in cell lines that differ from those directly tested here.

Table 1: Summary of the Rho GDI 1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Abcam	ab108977**	GR51596-7	AB_10890802	recombinant-mono	EPR3772	rabbit	0.23	Wb
Abcam	ab133248**	GR91858-7	AB_11157730	recombinant-mono	EPR3773	rabbit	0.02	Wb
Abcam	ab135252*	GR313436-12	AB_2893181	monoclonal	2G3	mouse	1.00	Wb,IF
Proteintech	10509-1-lg	636	AB_2923266	polyclonal	-	rabbit	0.60	Wb
Proteintech	66480-1-lg*	10004594	AB_2923267	monoclonal	1B1G9	mouse	1.50	Wb
Bio-Techne	NBP2-02467*	A001	AB_2923268	monoclonal	OTI1F2	mouse	1.00	Wb
GeneTex	GTX101428	39721	AB_1241259	polyclonal	-	rabbit	0.76	Wb
GeneTex	GTX60618*	822104509	AB_2923269	monoclonal	2G3	mouse	1.00	Wb,IF
GeneTex	GTX84860*	822104503	AB_10733320	monoclonal	1A7	mouse	0.72	Wb,IF
ABclonal	A11556**	40000000629	AB_2861595	recombinant-mono	ARC0629	rabbit	0.10	Wb
Cell Signaling Technology	2564	2	AB_2274313	polyclonal	-	rabbit	0.039	Wb,IF
Thermo Fisher Scientific	MA5-25090*	XD3563902	AB_2725231	monoclonal	OTI1A7	mouse	0.72	Wb,IF
Thermo Fisher Scientific	MA5-25081*	XD3563901	AB_2723577	monoclonal	OTI1F2	mouse	1.00	Wb,IF
Thermo Fisher Scientific	MA5-17032*	XD3563284	AB_2538504	monoclonal	2G3	mouse	1.00	Wb,IF

Wb=Western blot, IP= immunoprecipitation, IF=immunofluorescence, *=monoclonal antibody, **=recombinant antibody

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
Abcam	ab255449	CVCL_0063	HEK-293T	WT
Abcam	ab266446	CVCL_B2S1	HEK-293T	*ARHGDIA* KO

Figure 1: Rho GDI 1 antibody screening by immunoblot.

Lysates of HEK-293T WT and *ARHGDIA* KO were prepared, and 20 µg of protein were processed for immunoblot with the indicated Rho GDI 1 antibodies. The Ponceau stained transfers of each blot are shown. All tested antibodies were diluted at 1/1000. Predicted band size: 23 kDa. *=monoclonal antibody, **=recombinant antibody

Figure 2: Rho GDI 1 antibody screening by immunoprecipitation.

HEK-293T lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated Rho GDI 1 antibodies pre-coupled to Dynabeads protein G or protein A. Samples were washed and processed for immunoblot with the indicated Rho GDI 1 antibody. For immunoblot, A11556 was used at 1/1000. The Ponceau stained transfers of each blot are shown. SM=4% starting material; UB=4% unbound fraction; IP=immunoprecipitate; *=monoclonal antibody, **=recombinant antibody

Figure 3: Rho GDI 1 antibody screening by immunofluorescence.

HEK-293T WT and *ARHGDIA* KO cells were labelled with a green or a far-red fluorescent dye, respectively. WT and KO cells were mixed and plated to a 1:1 ratio in a 96-well plate with glass bottom. Cells were stained with the indicated Rho GDI 1 antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody including DAPI. Acquisition of the blue (nucleus-DAPI), green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) channels was performed. Representative images of the blue and red (grayscale) channels are shown. WT and KO cells are outlined with green and magenta dashed line, respectively. Antibodies were used at 1.0 µg/ml. Bars = 20 µm. *=monoclonal antibody, **=recombinant antibody

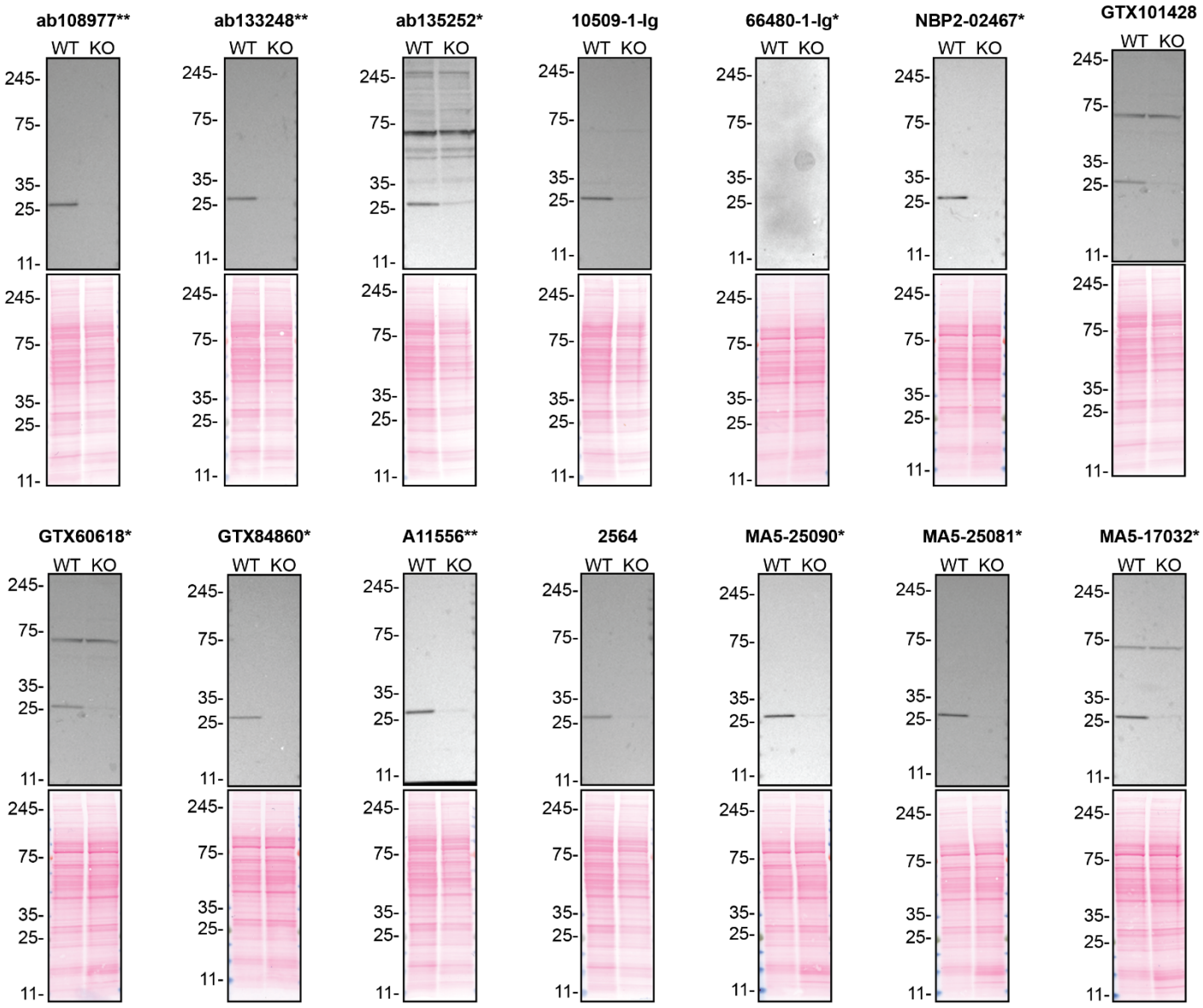

Figure 1: Rho GDI 1 antibody screening by immunoblot

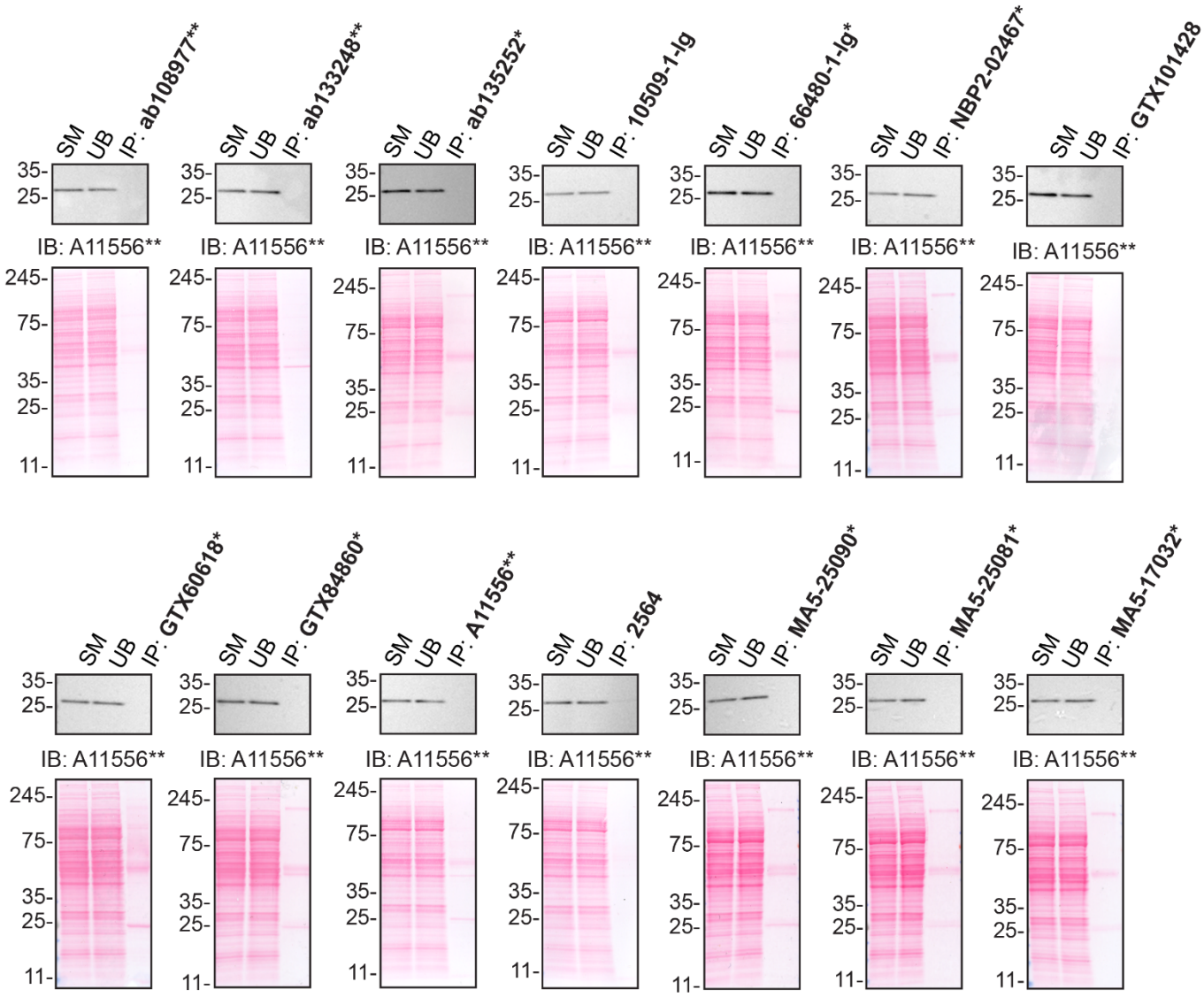

Figure 2: Rho GDI 1 antibody screening by immunoprecipitation

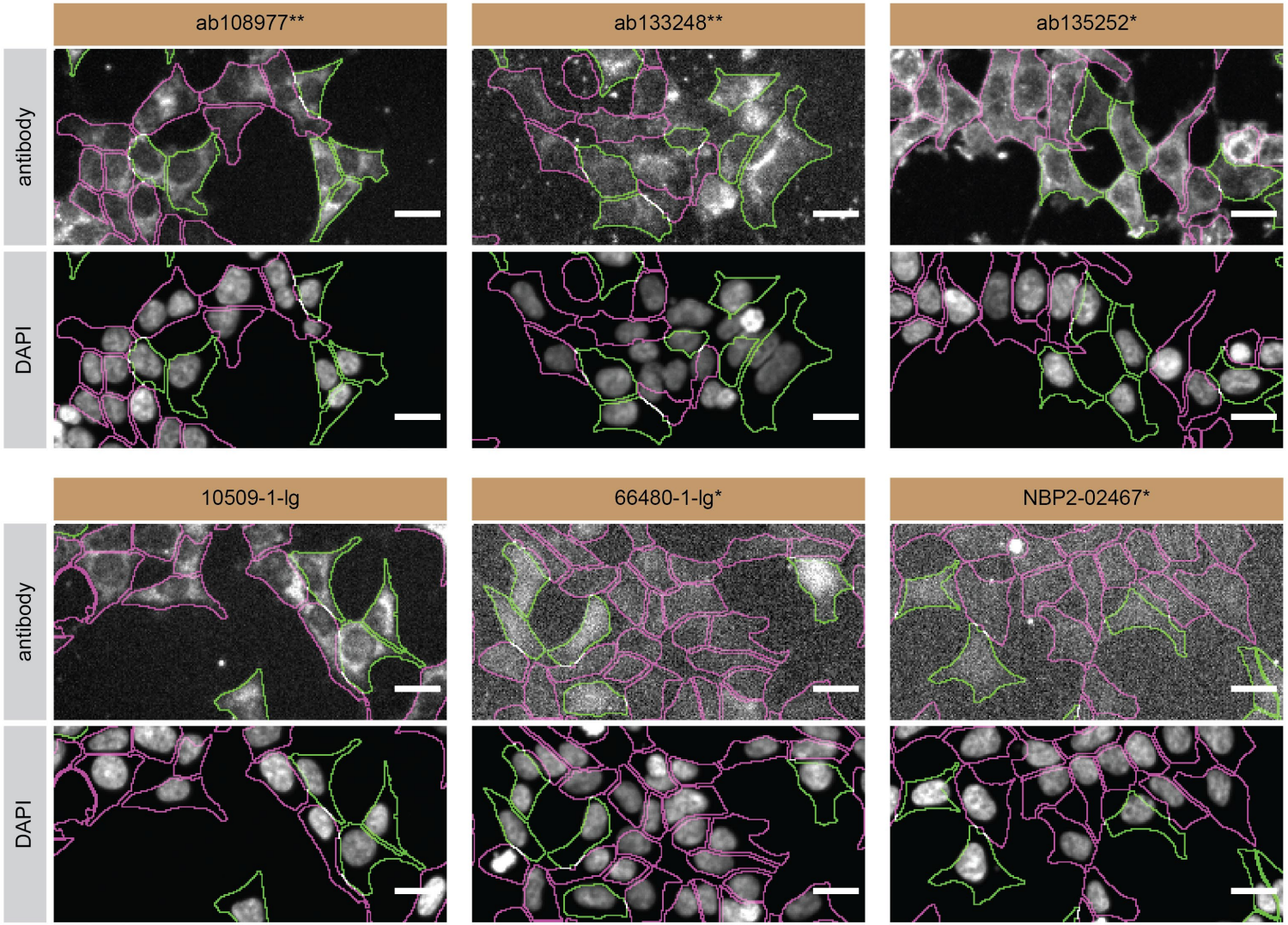

Figure 3: Rho GDI 1 antibody screening by immunofluorescence (1/3)

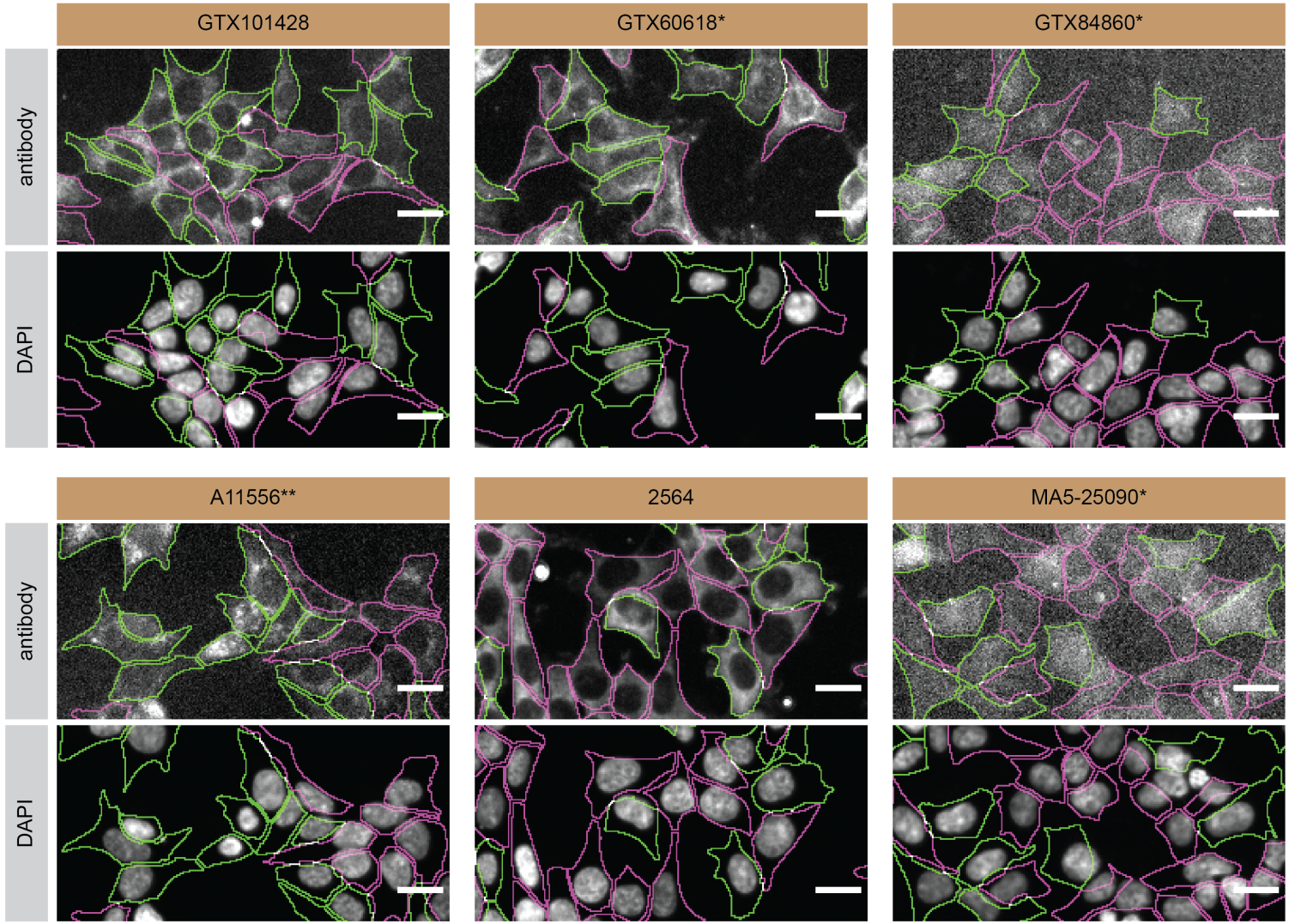

Figure 3: Rho GDI 1 antibody screening by immunofluorescence (2/3)

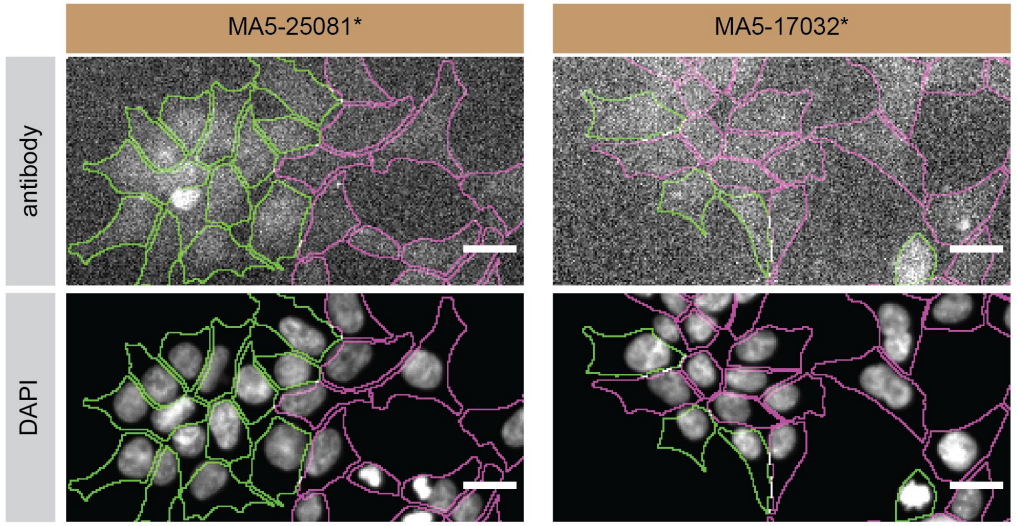

Figure 3: Rho GDI 1 antibody screening by immunofluorescence (3/3)

Materials and methods

Antibodies

All Rho GDI 1 antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse and anti-rabbit antibodies are from Thermo Fisher Scientific (cat. number 62-6520 and 65-6120). Alexa-555-conjugated goat anti-mouse and anti-rabbit secondary antibodies are from Thermo Fisher Scientific (cat. number A21424 and A21429).

Cell culture

Cells were cultured in DMEM high glucose (GE Healthcare cat. number SH30081.01) containing 10% fetal bovine serum (Wisent, cat. number 080450), 2 mM L-glutamate (Wisent cat. number 609065, 100 IU penicillin and 100 µg/ml streptomycin (Wisent cat. number 450201).

Antibody screening by immunoblot

Immunoblots were performed as described in our standard operating procedure [4]. HEK-293T WT and *ARHGDIA* KO were collected in RIPA buffer (25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor. Lysates were sonicated briefly and incubated 30 min on ice. Lysates were spun at ~110,000xg for 15 min at 4°C and equal protein aliquots of the supernatants were analyzed by SDS-PAGE and immunoblot. BLUelf prestained protein ladder from GeneDireX (cat. number PM008-0500) was used.

Immunoblots were performed with precast midi 4-20% gradient polyacrylamide gels from Thermo Fisher Scientific (cat. number WXP42012BOX) and transferred on nitrocellulose membranes. Proteins on the blots were visualized with Ponceau staining which is scanned to show together with individual immunoblot. Blots were blocked with 5% milk for 1 hr, and antibodies were incubated O/N at 4°C with 5% bovine serum albumin in TBS with 0,1% Tween 20 (TBST). Following three washes with TBST, the peroxidase conjugated secondary antibody was incubated at a dilution of ~0.2 µg/ml in TBST with 5% milk for 1 hr at room temperature followed by three washes with TBST. Membranes are incubated with Clarity Western ECL Substrate from Bio-Rad (cat. number 1705061) prior to detection iBright™ CL1500 Imaging System from Thermo Fisher Scientific (cat. number A44240).

Antibody screening by immunoprecipitation

Immunoprecipitation was performed as described in our standard operating procedure [5]. Antibody-bead conjugates were prepared by adding 2 µg to 500 ul of Pierce IP Lysis Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together with with 30µl of Dynabeads protein A- (for rabbit antibodies) or protein G- (for mouse antibodies) from Thermo Fisher Scientific (cat. number 10002D and 10004D, respectively). Tubes were rocked for ~2 hrs at 4°C followed by several washes to remove unbound antibodies.

HEK-293T WT were collected in Pierce IP buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol) supplemented with protease inhibitor. Lysates are rocked 30 min at 4°C and spun at 110,000xg for 15 min at 4°C. One ml aliquots at 1.0 mg/ml of lysate were incubated with an antibody-bead conjugate for ~2 hrs at 4°C. Following centrifugation, the unbound fractions were collected, and beads were subsequently washed three times with 1.0 ml of IP lysis buffer and processed for SDS-PAGE and immunoblot on midi 4-20% gradient polyacrylamide gels. Prot-A:HRP from MilliporeSigma (cat. number P8651) was used as a secondary detection system at a concentration of 0.3 µg/ml.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [6]. HEK-293T WT and *ARHGDIA* KO were labelled with a green and a far-red fluorescence dye, respectively. The fluorescent dyes used are from Thermo Fisher Scientific (cat. number C2925 and C34565). WT and KO cells were plated on glass coverslips as a mosaic and incubated for 24 hrs in a cell culture incubator. Cells were fixed in 4% PFA (in PBS) for 15 min at room temperature and then washed 3 times with PBS. Cells were permeabilized in PBS with 0,1% Triton X-100 for 10 min at room temperature and blocked with PBS with 5% BSA, 5% goat serum and 0.01% Triton X-100 for 30 min at room temperature. Cells were incubated with IF buffer (PBS, 5% BSA, 0,01% Triton X-100) containing the primary Rho GDI 1 antibodies O/N at 4°C. Cells were then washed 3 × 10 min with IF buffer and incubated with corresponding Alexa Fluor 555-conjugated secondary antibodies in IF buffer at a dilution of 1.0 µg/ml for 1 hr at room temperature with DAPI. Cells were washed 3 × 10 min with IF buffer and once with PBS.

Images were acquired on an ImageXpress micro confocal high-content microscopy system (Molecular Devices), using a 20x NA 0.95 water immersion objective and scientific CMOS cameras, equipped with 395, 475, 555 and 635 nm solid state LED lights (lumencor Aura III light engine) and bandpass filters to excite DAPI, Cellmask Green, Alexa568 and Cellmask Red,

respectively. Images had pixel sizes of 0.68 x 0.68 microns, and a z-interval of 4 microns. For analysis and visualization, shading correction (shade only) was carried out for all images. Then, maximum intensity projections were generated using 3 z-slices. Segmentation was carried out separately on maximum intensity projections of Cellmask channels using CellPose 1.0, and masks were used to generate outlines and for intensity quantification.

References

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